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Morphology and function of the ovipositor mechanism in Ceraphronoidea (Hymenoptera, Apocrita)

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Abstract

The ovipositor of apocritan Hymenoptera is an invaluable source of phylogenetically relevant characters, and our understanding of its functional morphology stands to enlighten us about parasitoid life history strategies. Although Ceraphronoidea is one of the most commonly collected Hymenoptera taxa with considerable economic importance, our knowledge about their natural history and phylogenetic relationships, both to other apocritan lineages and within the superfamily itself, is limited. As a first step towards revealing ceraphronoid natural diversity we describe the skeletomuscular system of the ceraphronoid ovipositor for the first time. Dissections and Confocal Laser Scanning Microscopy 3D media files were used to visualize the ovipositor complex and to develop character concepts. Morphological structures were described in natural language and then translated into a character-character state format, whose terminology was linked to phenotype-relevant ontologies. Four unique anatomical phenotypes were revealed: 1. The first valvifer (gonangulum) of the genus *Trassedia* is composed of two articulating sclerites, a condition present only in a few basal insect taxa. The bipartition of the first valvifer in *Trassedia* is most likely secondary and might allow more rapid oviposition. 2. Ceraphronoids, unlike other Hymenoptera, lack the retractor muscle of the terebra; instead the egg laying device is retracted by the seventh sternite. 3. Also unlike other Hymenoptera, the cordate apodeme and the anterior flange of the second valvifer are fused and compose one ridge that serves as the site of attachment for the dorsal and ventral T9-second valvifer muscles. Overall, the ceraphronoid ovipositor system is highly variable and can be described by discrete, distinguishable character states. However, these differences, despite their discrete nature, do not reflect the present classification of the superfamily and might represent parallelisms driven by host biology.

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Keywords
Skeletomusculature, second valvula, ovipositor sheath, *Aphanogmus*, *Ceraphron*, *Conostigmus*, *Megaspilus*, *Dendrocerus*, *Lagynodes*, Hymenoptera Anatomy Ontology, Phenotypic Quality Ontology, Spatial Ontology

Introduction

Ceraphronoidea has, until recently, been one of the most neglected groups of parasitic Hymenoptera, despite their ubiquity in the environment and fascinating life history strategies, which span from primary to quaternary parasitism (hyper-hyper-hyperparasitism) (Haviland 1920). Since the establishment of Ceraphronoidea as a superfamily (Masner 1956, Masner and Dessart 1967) significant revisionary work has addressed Lagynodinae (Dessart 1977, 1987), species of *Conostigmus* Dahlbom (Dessart 1997) and species groups of *Dendrocerus* Ratzeburg (Dessart 1972, Fergusson 1980, Dessart 1985, Dessart 1995a, Dessart 1999, Dessart 2001). Records of parasitism by ceraphronoids cover a wide range of hosts, from at least eight insect orders: Hemiptera, Thysanoptera, Hymenoptera, Neuroptera, Coleoptera, Trichoptera, Diptera and Mecoptera (Austin 1984, Chiu et al. 1981, Cooper and Dessart 1975, Dessart 1967, 1992, Dessart and Bournier 1971, Evans et al. 2005, Fergusson 1980, Ghesquiere 1960, Ishii 1937, Luhman et al. 1999, Muesebeck 1979, Priesner 1936, Sinacori et al. 1992). Two species, *Dendrocerus carpenteri* (Curtis), and *Dendrocerus aphidum* (Rondani), serve as models for research concerning resource use and allocation by parasitoids (Araj et al. 2006), mate location (Schwörer et al. 1999), development and host interactions (Marris et al. 2000), sex determination of offspring (Chow and Mackauer 1996), host discrimination (Chow and Mackauer 1999), and behavioral evolution in bio-control systems (Müller et al. 1997).

The phylogenetic placement of Ceraphronoidea remains uncertain despite the recent efforts to resolve the Hymenoptera tree of life (Heraty et al. 2011, Sharkey et al. 2011). Using expressed sequence tag (EST) data and limited exemplars, Sharanowski et al. (2010) placed Ceraphronoidea as sister to Evanioidea, but with low support. Heraty et al. (2011), using data from four genetic loci, placed Ceraphronoidea as sister to Stephanidae or Stephanidae + Orussidae, depending on the analysis. Vilhelmsen et al. (2010) found Ceraphronoidea to be sister to Megalyroidea within Evaniomorpha, based on morphological data. Sharkey et al. (2011) using molecular data and morphological data - based largely on Vilhelmsen et al. (2010) - also suggested a sister relationship between Ceraphronoidea and Megalyroidea.

The most comprehensive review on the classification of the superfamily has proposed by Dessart and Cancemi (1987). Although this classification served as a template for further studies (Masner 1993, Dessart 1995a, b, Mikó and Deans 2009) the phylogenetic base has never been challenged. The utility of traditionally used morphological characters in Ceraphronoidea classification seems to be limited (Dessart 1995b,

c, 2001) and with the discovery of the first ceraphronid wasp with well-developed pterostigma even the family level classification of the superfamily has been challenged recently (Mikó and Deans 2009). Our recent observations on the genus *Trassedia* provide additional evidence that the current classification of the superfamily needs to be revised (Mikó et al. in press). The genus was erected by Cancemi (1996), who classified the type species within Megaspilinae and considered it to be closely related to *Conostigmus*. The original classification is supported by two distinct character states: the presence of a fore wing pterostigma (which has subsequently been found in Ceraphronidae; Mikó and Deans 2009) and the presence of 11 antennomeres (Ceraphronidae have 10). Our observations of multiple specimens of *Trassedia luapi* reveal numerous morphological character states that are specific to Ceraphronidae (Mikó and Deans 2009, Mikó et al. in press): absence of mesotibial apical spur, absence of narrow sclerite anterior to synsternum, presence of Waterston's evaporatorium, and numerous male genitalia characters. Based on these differences the genus has recently been transferred to Ceraphronidae (Mikó et al. in press), a classification we follow in this study.

It is evident that a comprehensive study using both morphological and molecular characters is necessary for the reevaluation of Ceraphronoidea systematics. Since traditionally used morphological characters have been phylogenetically inconsistent the utilization of unexplored character systems, such as the male and female terminalia might offer additional data relevant to more robustly estimate the phylogenetic history of this group.

Despite the extensive descriptive work done on comparative morphology of the Hymenoptera female terminalia (Oeser 1961, Smith 1970, Le Ralec et al. 1996, Quicke et al. 1992, Quicke et al. 1994, Quicke et al. 1999, Vilhelmsen 2000, Vilhelmsen et al. 2001), the available morphological data on the ceraphronoid female terminalia are restricted to the distal region of the terebra (Quicke et al. 1994, Le Ralec et al. 1996) and the accessory glands (Höller et al. 1993). The skeletomuscular system of ceraphronoid ovipositor remained, until now, relatively unexplored. The main aim of the present study is to describe morphological diversity of the female terminalia in Ceraphronoidea and compare its anatomical structures with that of other Hymenoptera taxa giving special emphasize on the skeletomuscular system. With this study, we establish a baseline for further phylogenetic analyses of the superfamily using ovipositor characters.

Materials and methods

Specimens used in this study (Table 1) were identified by Andrew Ernst (AFE) and István Mikó (IM). Megaspilinae was represented in our study by *Megaspilus armatus* (Say), *Dendrocerus spissicornis* (Hellén) and *Conostigmus abdominalis* (Boheman); Lagynodinae (Megaspilidae) by *Lagynodes* sp. (AFE); and Ceraphronidae by *Ceraphron* sp. (IM) (Fig. 5), *Aphanogmus* sp.1 (AFE), *Aphanogmus* sp. 2 (IM) and *Trassedia luapi* (Cancemi).

Table 1. Specimens observed for morphological description including specimen identifiers, preparation techniques, imaging method and collecting locality (verbatim collecting data are stored at fighsare.com.).

Taxon	Specimen identifier	Imaging techniques	Locality	DOI of CLSM media files stored at http://figshare.com
Aphanogmus sp. 1	NCSU 0055648	brightfield	Hungary	
Aphanogmus sp. 1	NCSU 0002419	CLSM 40× water immersion	Hungary	doi: 10.6084/m9.figshare.156446 doi: 10.6084/m9.figshare.156439
Aphanogmus sp. 2	PSUCIM_5009	brightfield	Madagascar	
Ceraphron sp.	NCSU 0071198	brightfield	Madagascar	
Ceraphron sp.	NCSU 0071197	brightfield, CLSM 10x	Madagascar	doi: 10.6084/m9.figshare.156445
Ceraphron sp.	PSUCIM_5005, 5006	CLSM	Madagascar	doi: 10.6084/m9.figshare.156470 doi: 10.6084/m9.figshare.156469 doi: 10.6084/m9.figshare.156447
Conostigmus abdominalis (Boheman)	NCSU 0056302	brightfield	Sweden	
Conostigmus abdominalis (Boheman)	NCSU 0056301	brightfield	Sweden	
Conostigmus abdominalis (Boheman)	NCSU 0055647	brightfield, CLSM 20×	Sweden	doi: 10.6084/m9.figshare.156448 doi: 10.6084/m9.figshare.156433
Dendrocerus spissicornis Hellén	PSUCIM_5001, 5002	CLSM	Sweden	doi: 10.6084/m9.figshare.156452 doi: 10.6084/m9.figshare.156451 doi: 10.6084/m9.figshare.156450 doi: 10.6084/m9.figshare.156449
Dendrocerus spissicornis Hellén	PSUCIM_5010	CLSM	Sweden	doi: 10.6084/m9.figshare.156459 doi: 10.6084/m9.figshare.156453
Lagynodes sp.	NCSU 0055643	brightfield	USA	
Lagynodes sp.	NCSU 0056306	CLSM 20×, 40× water immersion	USA	doi: 10.6084/m9.figshare.156454 doi: 10.6084/m9.figshare.156443
Lagynodes crassicornis				
Megaspilus armatus (Say)	NCSU 0071199	brightfield	USA	
Megaspilus armatus (Say)	NCSU 0055645	brightfield, CLSM 20×	USA	doi: 10.6084/m9.figshare.156442 doi: 10.6084/m9.figshare.156434
Megaspilus armatus (Say)	NCSU 0056307	CLSM 40× water immersion	USA	doi: 10.6084/m9.figshare.156437
Megaspilus armatus	PSUCIM_5003	CLSM 20X	Canada	doi: 10.6084/m9.figshare.156458 doi: 10.6084/m9.figshare.156457 doi: 10.6084/m9.figshare.156456 doi: 10.6084/m9.figshare.156455
Trassedia luapi Cancemi	NCSU 0056318	brightfield, CLSM 10×	Madagascar	doi: 10.6084/m9.figshare.156440 doi: 10.6084/m9.figshare.156438 doi: 10.6084/m9.figshare.156436
Trassedia luapi Cancemi	NCSU_0071196	brightfield, CLSM 20×	Madagascar	doi: 10.6084/m9.figshare.156467 doi: 10.6084/m9.figshare.156444

Specimens used in the present study were stored in 95% ethanol. Some specimens were critical point dried and dissected on Blue-Tack (Blue Tack, Bostik inc.) medium. This method is mostly used to reveal the spatial relationships between muscles. Other specimens were dissected in glycerin on a concave microscope slide or were macerated in KOH to visualize the skeletal structures. Dissections and observations were made using an Olympus SZX16 stereomicroscope and an Olympus CX41 compound microscope.

Bright field images were taken using an Olympus CX41 compound microscope, equipped with an Olympus DP71 digital camera. Image stacks were combined using CombineZP (Hadley 2010) "do stack" command. SEM micrographs were made using a Hitachi S-3200 Scanning Electron Microscope (wd=23.5, av=5kV). Specimens were critical point dried and coated with palladium prior to examination. CLSM images were made on glycerin-stored specimens between 1.5 mm thick, 24×50 mm cover glasses with a Leica LSM 710 and Olympus Fluoview 1000 confocal laser scanning microscopes (CLSM) using the 488 nm laser to excite the sample. We collected the autofluorescence of insect anatomical structures between 500 and 700 nm with two channels (500–580; pseudocolor green and 580–700 pseudocolor red) using 106 and 206 Plan Achromat objectives. Volume rendered images and media files were generated by Imaris Bitplane (Bitplane, Zürich, Switzerland) and ImageJ (Schneider et al. 2012) software. Bright field and CLSM micrographs were edited with Adobe Photoshop CS4 (Adobe) changing "Gamma Correction" value, resize images to 7.3 cm width at 400 dpi resolution, standardize style and width of scale bars and create image annotations. Media files, SEM micrographs and bright field images are available from figshare.com (Table 1).

To verify the relationships between anatomical structures, serial transverse sectioning was carried out on a *Lagynodes* specimen (Table 1). The specimen was embedded in Araldit®, cut at 1 μm with a Microm microtome (HM 360), and stained with toluidine blue.

Anatomical terms used in the descriptions are linked to concepts in the Hymenoptera Anatomy Ontology (HAO; Hymenoptera Anatomy Portal (http://portal.hymao.org), Yoder et al. 2010), the Phenotypic Quality Ontology 1.2 (PATO; Gkoutos et al. 2004) and the Biospatial Ontology (Mungall 2013) via a table of uniform resource identifiers (URIs) (Appendix) following Seltmann et al. 2012.

Towards semantic statements

Verbatim descriptions composed in natural language serve as the traditional way for communicating observations in insect morphology. However, these descriptions can be decoded only by morphology experts, are hardly accessible to non-expert researchers and cannot be reasoned over efficiently by text-mining applications (Vogt et al. 2010, Deans et al. 2012a, b, Vogt et al. 2013). Morphological descriptions, albeit they are arguably more complex, shares numerous similarities with taxonomic descriptions which

were the objects of recent efforts for altering the way of biodiversity descriptions into a more accessible format. Deans et al. (2012a) proposed a new description model for taxonomists applying semantic statements. These statements are written in a logic and queryable format and are linked to the concepts of biomedical ontologies. Semantic descriptions are therefore not only transparent for researchers unfamiliar with specific morphological jargon, but can be executed via an automated reasoning mechanism (Balhoff et al. in press, Mullins et al. 2012).

To meet the grand challenge of describing phenotypes in a semantic way, using Web Ontology Language (OWL; http://www.w3.org/TR/owl-features/) for example, one must be familiar with tools of the Semantic Web (e.g., Protégé, http://protege.stanford.edu/ and Manchester Syntax, http://www.w3.org/TR/owl2-manchester-syntax/). Perhaps more importantly, one also has to provide a character/character state description with terms explicitly linked to ontologies.

We provide here an example of the transformation of our natural language descriptions to character/character state format and to link the terminology to relevant phenotype ontologies. Our goal is to make this product more accessible to future reasoning applications. During the "ontologization" procedure the describer is forced to provide strict, structure-based definitions for each anatomical concept, which itself enhances the readability, objectivity, consistency and comparability of the research product.

Results I: Natural language descriptions of anatomical structures in the ceraphronoid ovipositor assembly and S7

Integument

The first valvifer is dorsoventrally elongated in lateral view (ch1: 0; **1vf**: Figs 2E, 5A, E, 6A, D) with convex anterior (ch2:0) and straight (ch3:0; **p1v**: Figs 2D, 4A, 6A) or concave posterior margins (ch3:1; **p1v**: Fig. 3D) in all taxa except *Dendrocerus* where the posterior margin of the first valvifer is angled at the tergo-valviferal articulation (ch3:2; **p1v**: Fig. 4F). The first valvifer is not subdivided (ch4:1; **1vf**: Figs 2E, 5A, E, 6A, D) except in *Trassedia*, where the transvalvifer conjunctiva (ch4:0; **tvc**: Fig. 3E) separates the elongate dorsal sclerite (ch5:0; **d1vf**: Fig. 3E) from the triangular ventral sclerite of the first valvifer (ch6:0; **v1vf**: Fig. 3E). The sclerites articulate with one another at the intravalvifer articulation (ch7:0; **iava**: Figs 3C, E), which is located anteriorly on the border between the two sclerites (ch8:0). The ventral margin of the dorsal sclerite (ch9:0) and the anterodorsal margin of the ventral sclerite are thickened relative to surrounding regions (ch10:0). The anterior flange of the first valvifer (**af1**: Fig. 1F) overlapping the second valvifer is present (ch11:0) in *Conostigmus, Dendrocerus* and *Megaspilus*, and *Lagynodes* but absent from Ceraphronidae (ch11:1). The first valvifer articulates with the second valvifer on its posteroventral corner (ch12:0; intervalvifer articulation, **iva**: Figs 1B, 2C, 3A–C, E, 4A, F, 5A, B, E, 6A, C, D) and with T9 on its posterior margin (ch13:0; tergo-valvifer articulation, **tva**: Figs 1B, 2C, 3B, C, E, 4A,

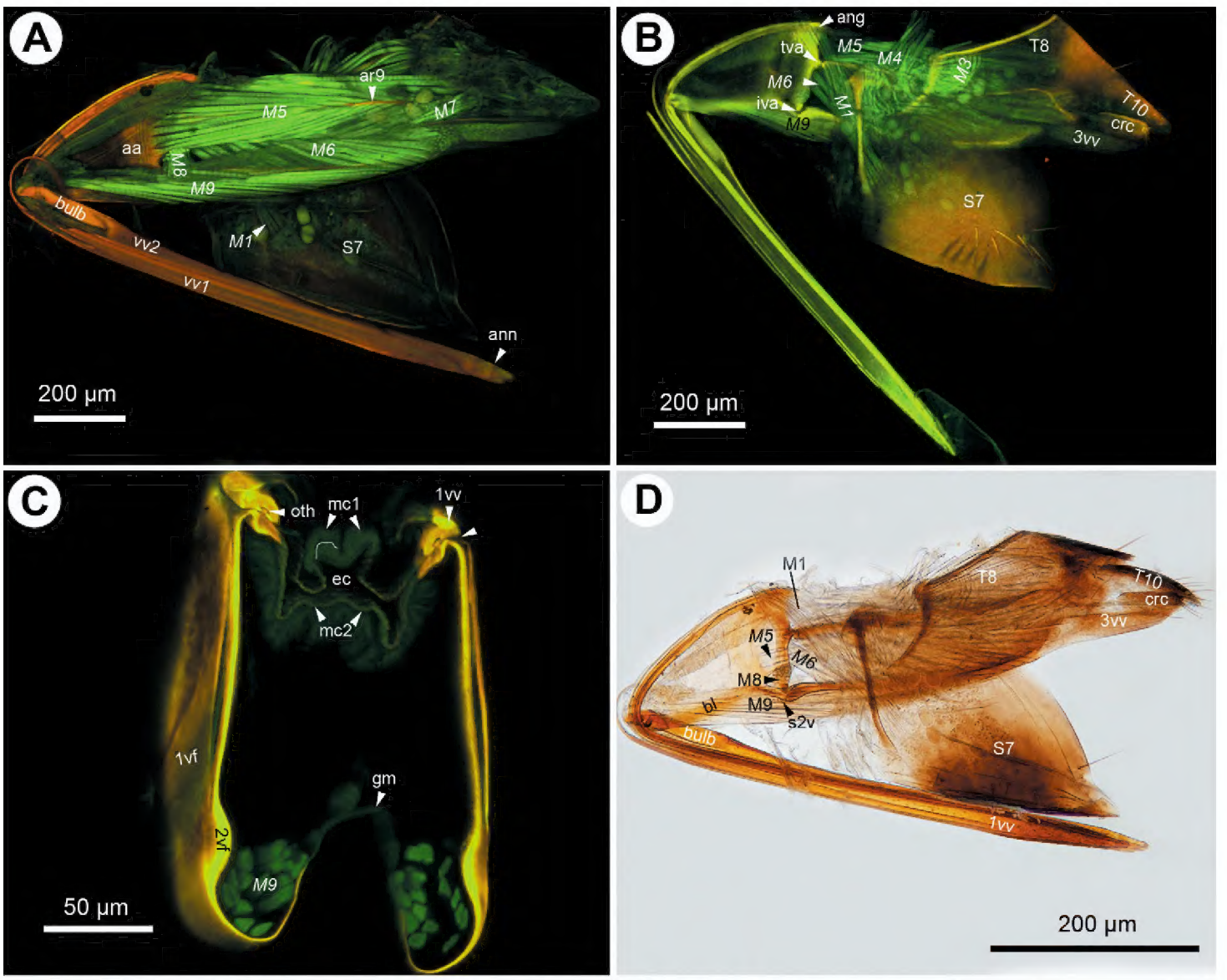

Figure 1. CLSM micrographs and bright field image showing the female terminalia of *Megaspilus armatus* (Say). **A** CLSM, medial view (doi: 10.6084/m9.figshare.156434) **B** CLSM, lateral view (doi: 10.6084/m9.figshare.156442) **C** CLSM, transverse section, white line indicates site of separation of median conjunctivae of the first valvulae (doi: 10.6084/m9.figshare.156437) **D** bright field, lateral view **E–F** CLSM, frontal section, dorsal view (doi: 10.6084/m9.figshare.156458; doi: 10.6084/m9.figshare.156457, doi: 10.6084/m9.figshare.156456, doi: 10.6084/m9.figshare.156455). All anterior to the left.

F, 5A, B, E, 6A, C, D). The tergo-valvifer articulation is in the middle of the posterior margin of the first valvifer in *Conostigmus* and *Lagynodes* (ch14:0; **tva**: Figs 2C, 4A), it is on the ventral half of the margin (ch14:1; **tva**: Figs 3B, C, E, 5A, B, E, 6D) in *Ceraphron*, *Aphanogmus* sp. 2 and *Trassedia,* where it is located ventrally on the posterior margin of the dorsal sclerite of the first valvifer (ch15:0; **tva**: Fig. 3B, C, E), it is in the upper half of the margin in *Megaspilus* and *Dendrocerus* (ch14:2; **tva**: Figs 1B, 4F) and adjacent to the anterior angle in *Aphanogmus* sp. 1 (ch14:3; **tva**: Figs 6A, C).

The anterior area of the second valvifer is expanded dorsally into a broad, flat, laterally directed surface (ch16:0; **aa**: Figs 1A, 2E). The basal line of the second valvifer (ch17:0) is a sharply defined ridge in *Ceraphron, Trassedia*, and *Aphanogmus* sp. 2 (ch18:0; **bl**: Figs 3B–D, 5A, E, 6D) but a thickening with diffuse margins in *Aphanogmus sp.1* and Megaspilidae (ch18:1; **bl**: Figs 1D, 2C, E, 4A, F). The dorsal projection of the second valvifer (ch19:0) is longer than the length of the anterior area of the second valvifer in *Ceraphron* and *Trassedia*, and *Aphanogmus* sp. 2 (ch20:1; **dp**: Figs 3C, E) whereas the projection is shorter than the length of the anterior area in other taxa examined (ch20:2. **dp**: Figs 2E, 4A). The anterior section of the dorsal flange of the second valvifer is sharply defined ridge in *Ceraphron*, *Aphanogmus* sp. 2, and *Trassedia* (ch21:0; **asf**: Figs 3B, C, D, 5A–E, 6D) but a thickening with diffuse margins in Megaspilidae and *Aphanogmus* sp. 1 (ch21:1; **asf**: Figs 2C, E, F, 4A, F). The posterior section of the dorsal flange of the second valvifer is sharply defined (ch22:0; **psf**: Figs 2C, E, 4A, D, 5A, E). The area of the second valvifer posterior to the intervalviofer articulation is elongate (ch23:0 **pa**: Figs 2C, E). The ventral margin of the second valvifer curves mediodorsally (ch24:0) encircling the posterior second valvifer-second valvula muscle (ch25:0. **2vf, M9**: Fig. 1C). The genital membrane of the second valvifers accommodates the terebra when it is retracted (ch26:0; **gm**: Fig. 1C). The venom gland reservoir is surrounded by the second valvifer is present in Ceraphronidae and *Lagynodes* (ch27:0; **res**: Figs 3A–E). The reservoir was not observed in other Megaspilidae (ch27:1). The content of the reservoir is yellowish, transparent and hard, resin-like (ch28:0; **res**: Fig. 5F) in critical point dried specimens.

The first valvula tapers distally in lateral view in *Ceraphron, Aphanogmus sp. 2* and *Trassedia* (ch29:0; **1vv**: Figs 3B, 5A, F, 6D), whereas it is spatulate in Megaspilidae and *Aphanogmus* sp. 1 (ch29:1; **1vv**: Figs 1A, D, 2A, C, D, E, 4A, B, 6B, C). The banding pattern and annuli are missing from the first valvula (ch30:0; ch31:0).

The second valvulae are expanded proximally into the bulb (ch37:0; **bulb**: Figs 1A, D) and fused distal to the bulb (ch35:0). The dorsal valve tapers distally in dorsal view (ch36:0) and the anterior margin of the bulb is curved dorsally. The processus articularis is located laterally (ch32:0; **pra**: Figs 2F, 5D), the anterior notch of the dorsal valve anteriorly (ch33:0) and the processus muscularis anterodorsally on the bulb (ch34:0; **prm**: Fig. 2F). The anterior area of the second valvifer is 2.0 times as high as the bulb in lateral view in *Dendrocerus* and *Aphanogmus* sp. 1 (ch38:0; Figs 4E, 6B) whereas it is more than 2.0 times as high as the bulb in *Ceraphron* and Megaspilidae (ch39:1). Banding pattern is absent (ch39:0) whereas annuli are present on the dorsal valve in Megaspilidae (ch40:0; **ann**: Figs 1A, 2C) but absent in Ceraphronidae (ch40:1). The

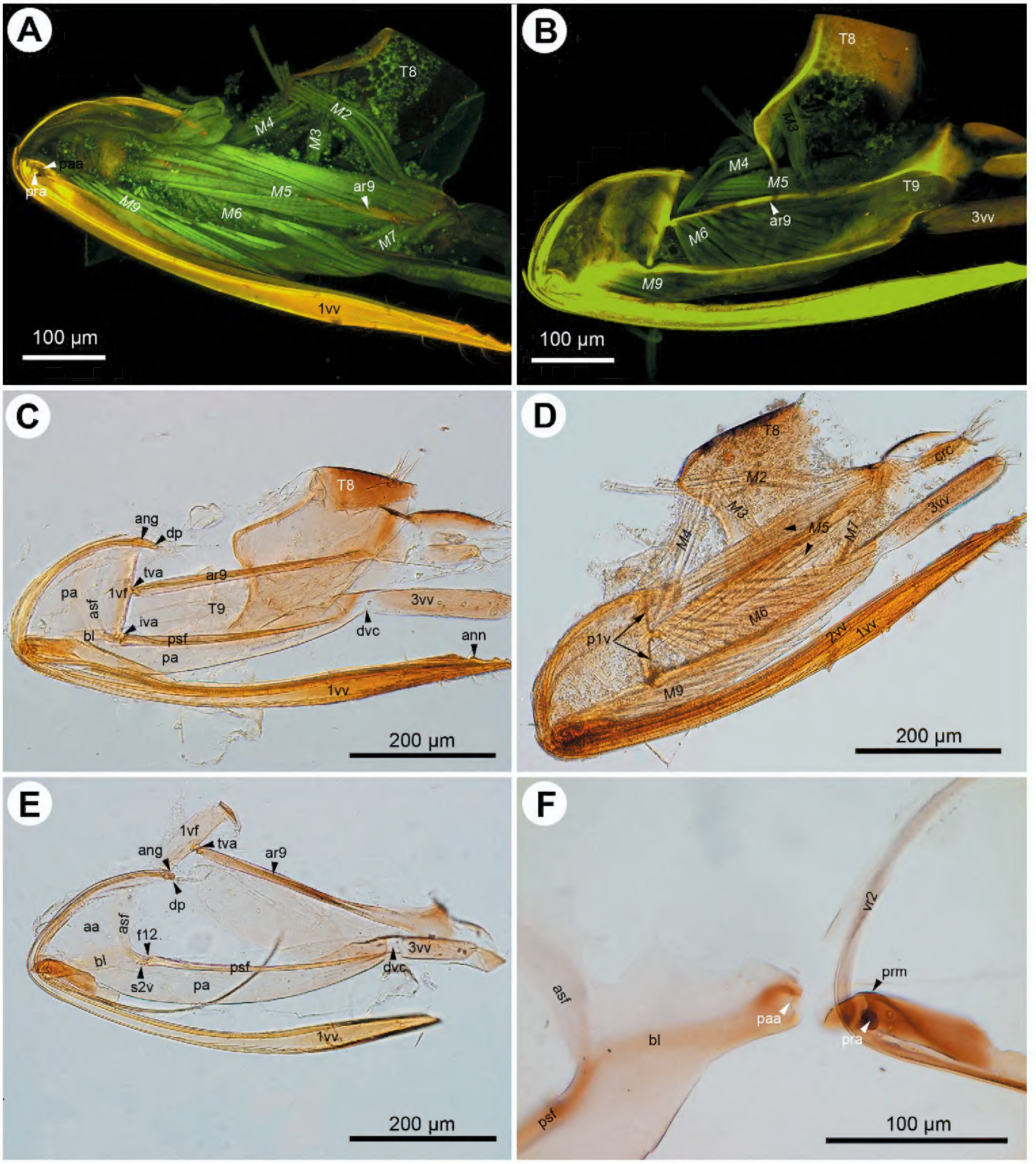

Figure 2. CLSM micrographs and bright field image showing the female terminalia of *Conostigmus abdominalis* (Boheman). **A** CLSM, medial view (doi: 10.6084/m9.figshare.156448) **B** CLSM, lateral view (doi: 10.6084/m9.figshare.156433) **C** bright field, medial view, muscles removed **D** bright field, medial view **E** bright field, medial view, first valvifer and T9 separated from second valvifer, muscles removed; F, bright field, second valvifer removed from second valvulae, muscles removed. All anterior to the left.

number of annuli is four (ch41:0) in *Conostigmus* and *Lagynodes* and six (ch41:1) in *Megaspilus*.

The third valvula and the second valvifer are fused at the median bridge (ch42:0; ch44:0; **mbr**: Figs 3C, E) connecting the opposite second valvifers at their posterior ends. A ventral, vertical conjunctiva marks the site of fusion of the two sclerites (ch43:1; **dvc**: Figs 2C, E, 3D, E, 4D). The third valvula tapers distally in lateral view

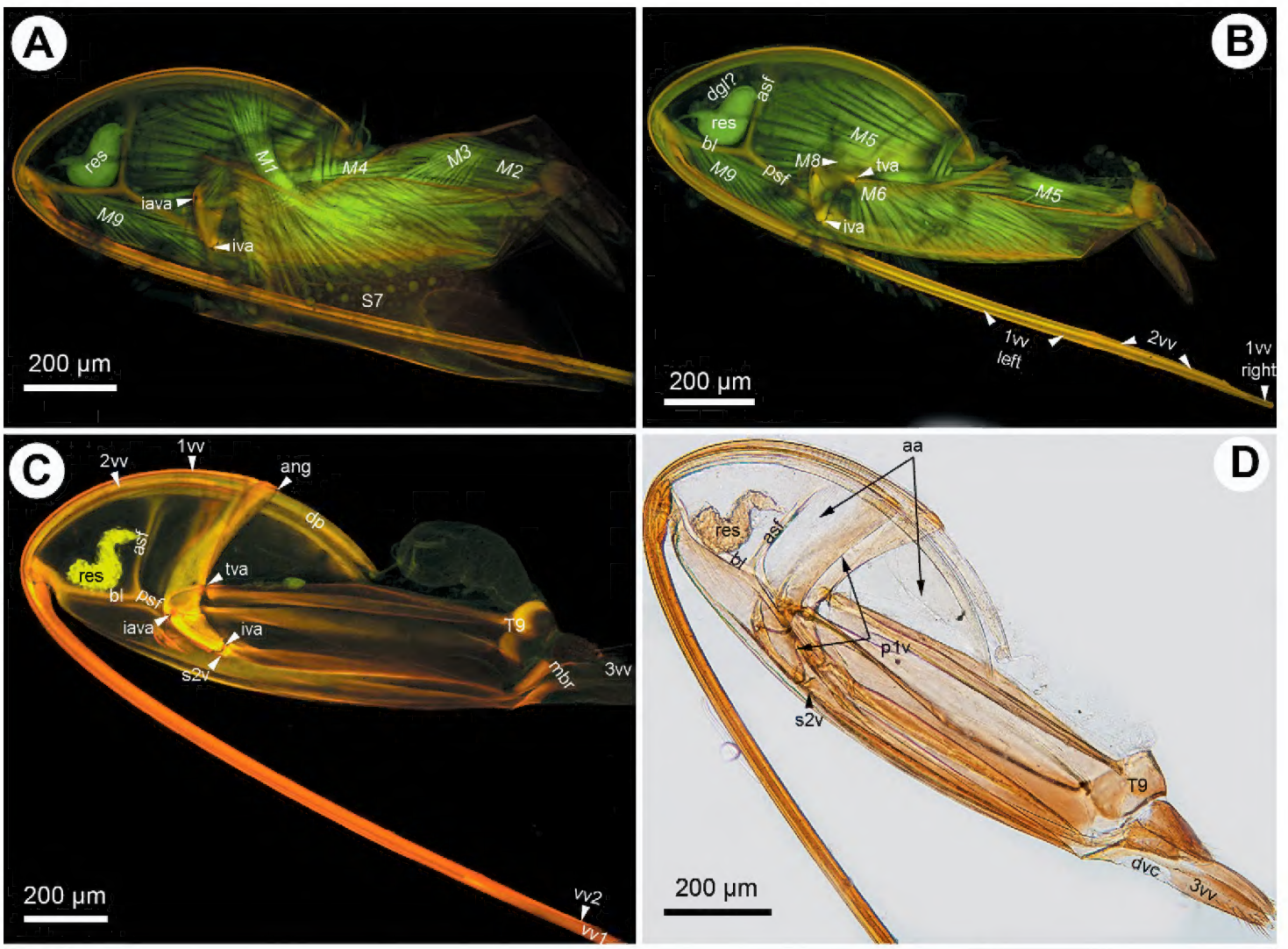

Figure 3. CLSM micrographs and bright field image showing the female terminalia of *Trassedia luapi* (Cancemi). **A** CLSM, lateral view, T8 intact (doi: 10.6084/m9.figshare.156440) **B** CLSM, lateral view, T8 removed (doi: 10.6084/m9.figshare.156438) **C** CLSM, lateral view, muscles removed (doi: 10.6084/m9.figshare.156444) **D** bright field, lateral view, muscles removed **E** CLSM, lateral view, muscles removed (doi: 10.6084/m9.figshare.156467). All anterior to the left.

(ch45:0; **3vv**: Figs 1B, 2B–E, 3C–E, 4D, F, 5E, F, 6A, B, F), its lateral wall is convex (ch46:0) and sclerotized (ch47:0) whereas its medial wall is concave (ch48:0; **3vv**: Fig. 3E) and membranous (ch49: 0; **3vv**: Fig. 3F).

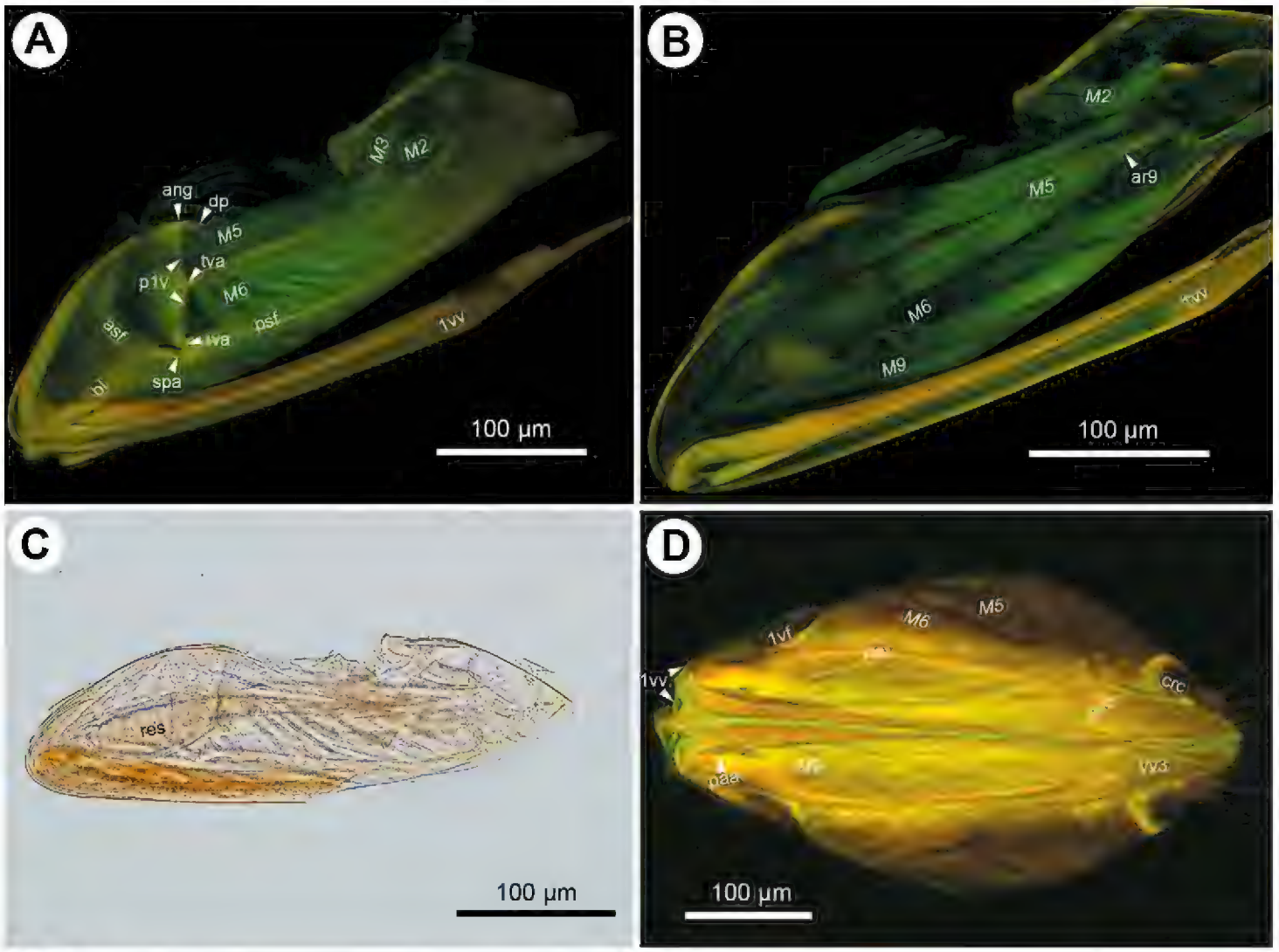

Figure 4. CLSM micrographs and bright field image showing the female terminalia of Megaspilidae. A–C: *Lagynodes* sp.: **A** CLSM, lateral view (doi: 10.6084/m9.figshare.156443) **B** CLSM, medial view (doi: 10.6084/m9.figshare.156454) **C** bright field, medial view. **D–F** *Dendrocerus spissicornis* (Hellén), CLSM: **D** ventral view (doi: 10.6084/m9.figshare.156451, doi: 10.6084/m9.figshare.156449) **E** medial view (doi: 10.6084/m9.figshare.156452) **F** lateral view (doi: 10.6084/m9.figshare.156459, doi: 10.6084/m9.figshare.156450). All anterior to left.

T9 is quadrangular in lateral view (ch50:0; T9: Figs 2B, C) and wider than long dorsomedially (ch51:0; T9: Figs 3D, E). The cordate apodeme and the anterior flange are continuous, composing the anterior ridge of T9 (ch52:0; **ar9**: Figs 2A–C, 3E, 4B), which is adjacent anteriorly with and separated posteriorly from the anterior margin of T9 (ch53:0.); the distance between the anterior margin of T9 and the ridge increases gradually posteriorly (ch54:0).

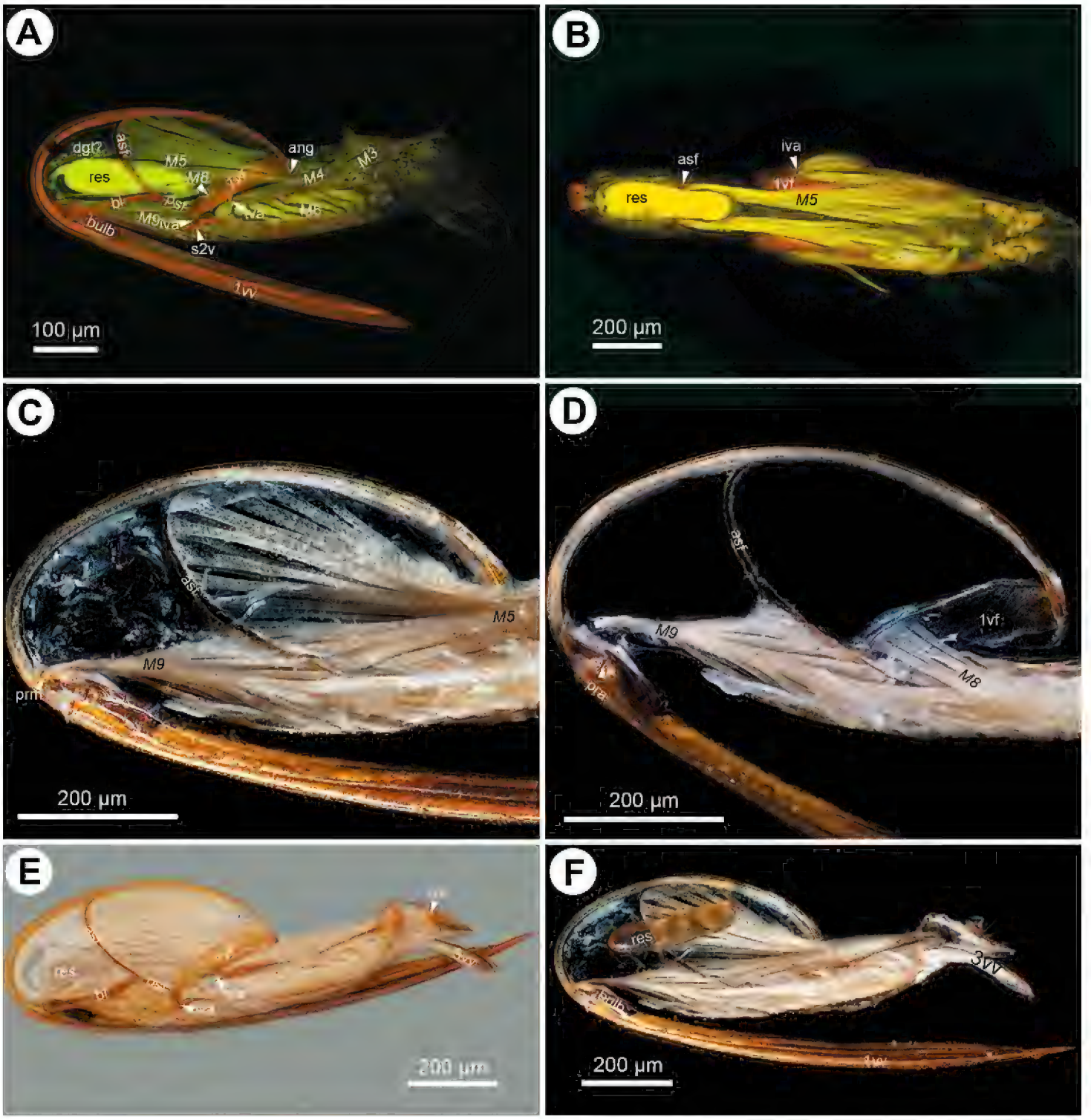

Figure 5. CLSM micrographs and bright field image showing the female terminalia of *Ceraphron* sp. **A** CLSM, lateral view (doi: 10.6084/m9.figshare.156445) **B** CLSM, frontal section, dorsal view (doi: 10.6084/m9.figshare.156447) **C** bright field, lateral view **D** bright field, medial view, dorsal T9-second valvifer muscle removed **E** bright field, medial view, dorsal T9-second valvifer muscle intact; F, bright field, medial view, venom gland reservoir intact. All anterior to left.

Muscles (numbered according to Vilhelmsen 2000)

S7-first valvula muscle (**M1**: Figs 1B, D, 3A) arises anteriorly from S7 (ch55:0) and inserts on the first valvula at the dorsal margin of the ovipositor adjacent to the border of the first valvula and the dorsal end of the first valvifer (ch56:0). The muscle is oriented posterodorsally (ch57:0). The dorsal T8-T9 muscle (**M2**: Figs 2A, 3A, 4A, B) arises from along the anterior margin of T8 (ch58:0) and inserts dorsally on the anterior margin of T9 (ch59:0). The lateral T8-T9 muscle (**M3**: Figs 1B, 2A, B, D,

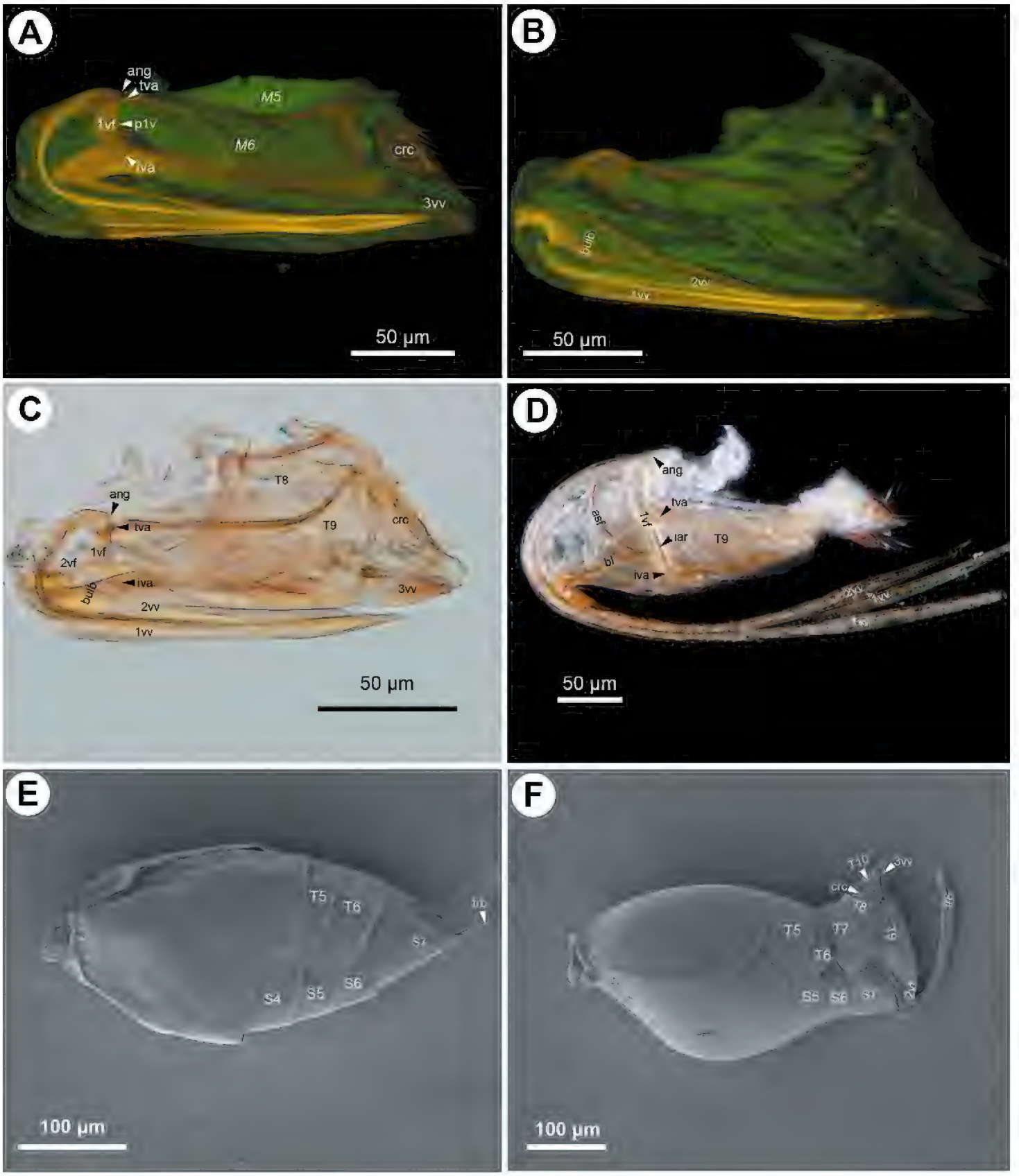

Figure 6. CLSM micrographs and bright field image showing the female terminalia of Ceraphronidae. **A–C**: *Aphanogmus* sp. 1: **A** CLSM, lateral view (doi: 10.6084/m9.figshare.156439) **B** CLSM, medial view (doi: 10.6084/m9.figshare.156446) **C** bright field, lateral view **D** *Aphanogmus* sp. 2, bright field, lateral view **E** *Synarsis* sp., SEM, lateral view **F** *Cyoceraphron* sp., SEM, lateral view. All anterior to left.

3A, 4A, 5A) arises from T8 posterodorsally of the dorsal T8-T9 muscle (ch60:0), and inserts on the anterior ridge of T9 (ch61:0). The T8-first valvifer muscle (**M4**: Figs 1B, 2A, 2B, 3A, 5A) arises from T8 dorsally of the site of attachment of the lateral T8-T9 muscle (ch62:0) and inserts on the first valvifer adjacent to the ninth tergal condyle of the first valvifer (ch63:0). The muscle inserts on the ventral sclerite of the first valvifer adjacent to the intravalvifer articulation (ch64:0; **M4**: Fig. 3A)

in *Trassedia*. The dorsal T9-second valvifer muscle (**M5**: Figs 1A, B, D, E, 2A, B, D, 3B, 4A, B, D, E, F, 5A–C, 6A) arises dorsally and ventrally from the anterior ridge of T9 with sites of origins extending ventrally and dorsally on the wall of the tergite (ch65:0) and inserts along the posterior margin of the anterior area of the second valvifer (ch66:0). The ventral T9-second valvifer muscle (**M6**: Figs 1A, B, D, E, 2A, B, D, 3B, 4A, B, D, F, 5A, B, 6A) is not subdivided (ch67:0) and arises from the medial side of the posterior area of the second valvifer (ch68:0). The site of insertion of the muscle extends along the anterior part of the anterior ridge of T9 in most taxa examined (ch69:0) except *Megaspilus*, and *Dendrocerus* where the muscle seemingly inserts partly on the interarticular ridge of the first valvifer (ch70:0; **M6**: Figs 1B, D, 4F). The posterior T9-second valvifer muscle (**M7**: Figs 1A, 2A, D, 4E) arises from the strap-like dorsal area of T9 (ch71:0) and inserts on the median bridge (ch72:0). The fan shaped first valvifer-genital membrane muscle (ch73:0; **M8**: Figs 1A, D, E, F, 3B, 5A, D) arises from the medial surface of the first valvifer near the intervalvifer articulation (ch74:0.). The site of insertion of the muscle extends along the genital membrane (ch75:0). In *Trassedia* the muscle arises from the medial surface of the dorsal sclerite of the first valvifer (ch76:1; **M8**: Fig. 3B). The posterior second valvifer-second valvula muscle (**M9**: Figs 1A–F, 2A, B, D, 3A, B, 4B, D–F, 5A, C, D) arises from the medial side of the posterior area of the second valvifer (ch77:0), and inserts on the processus musculares (ch78:0). In *Ceraphron and Trassedia* some bands of the muscle arise from the anterior area of the second valvifer (ch.77:1; **M9**: Figs 3A, B, 5A, C, D). T9-genital membrane muscle (ch79:0), lateral T9-second valvifer muscle (ch80:0), second valvifer-genital membrane muscle (ch81:0) and the anterior second valvifer-second valvula muscles (ch82:0) are absent from Ceraphronoidea.

Results II: Semantically enhanced characters and character states for ceraphronoid female terminalia.

Integument

First valvifer: shape
- (0) elongated in lateral view

Anterior margin of first valvifer: shape
- (0) convex in lateral view

Posterior margin of first valvifer: shape
- (0) straight in lateral view
- (1) concave in lateral view
- (2) bent at tergo-valvifer articulation

Transvalvifer conjunctiva: count
- (0) present
- (1) absent

Dorsal sclerite of the first valvifer: shape
(0) elongated in lateral view
Ventral sclerite of the first valvifer: shape
(0) triangular in lateral view
Intravalvifer articulation: count
(0) present
(1) absent
Intravalvifer articulation: position
(0) anterior region of first valvifer
Ventral margin of dorsal sclerite of the first valvifer: thickness
(0) thickened
Anterodorsal margin of ventral sclerite of the first valvifer: thickness
(0) thickened
Anterior flange of the first valvifer: count
(0) present
(1) absent
Second valviferal condyle of the first valvifer: position
(0) at the posteroventral corner of the first valvifer
Ninth tergal condyle of the first valvifer: position
(0) posterior margin of the first valvifer
Distance between tergo-valvifer articulation and intravalvifer articulation (D1): proportion to the distance between tergo-valvifer articulation and anterior angle of first valvifer (D2).
(0) D1=D2
(1) D1<D2
(2) D1>D2
(3) D2=0
Ninth tergal condyle of the first valvifer: position
(0) on dorsal sclerite of the first valvifer
Anterior area of the second valvifer: height vs. height of posterior area of second valvifer
(0) 3≤:1
Basal line of the second valvifer: count
(0) present
Basal line of the second valvifer: sharpness
(0) sharp
(1) blunt
Dorsal projection of the second valvifer: count
(0) present
Length of dorsal projection of second valvifer in lateral view (L1) vs. length of anterior area of second valvifer in lateral view (L2)
(0) L1<L2
(1) L1>L2

Anterior section of dorsal flange of the second valvifer: sharpness
(0) sharp
(1) blunt
Posterior section of dorsal flange of second valvifer: sharpness
(0) sharp
Posterior area of second valvifer: shape
(0) elongated
Ventral margin of the second valvifer: curvature
(0) curved medially and curved dorsally in lateral view
Ventral margin of the second valvifer: position
(0) surrounds posterior second valvifer-second valvula muscle
Genital membrane: position
(0) surrounds terebra
Venom gland reservoir of the second valvifer: count
(0) present
(1) absent
Venom gland reservoir of the second valvifer: physical quality
(0) resinous
Distal region of first valvula: shape
(0) tapered
(1) spatulate
Banding pattern on first valvula: count
(0) absent
Annuli on first valvula: count
(0) absent
Processus articularis: position
(0) anterolateral region of the bulb
Anterior notch of the dorsal valve: position
(0) anterior region of the bulb
Processus musculares: position
(0) anterodorsal region of the bulb
Distal notch of the dorsal valve: count
(0) absent
Distal region of the dorsal valve: shape
(0) tapered in dorsal view
Anterior region of dorsal margin of the bulb: curvature
(0) curved dorsally in lateral view
Bulb: height in lateral view vs. height of anterior area of the second valvifer in lateral view
(0) =0.5
(1) <0.5
Banding pattern on dorsal valve: count
(0) absent

Annuli on dorsal valve: count
(0) present
(1) absent
Annuli on dorsal valve: count value
(0) four
(1) six
Median bridge: count
(0) present
Distal vertical conjunctiva of the second valvifer-third valvifer complex: count
(0) present
Distal vertical conjunctiva of the second valvifer-third valvifer complex: overlap relationship with dorsal margin of second valvifer-third valvula complex
(0) does not overlap
Distal region of the third valvula: shape
(0) tapered in lateral view
Lateral region of the third valvula: shape
(0) convex in posterior view
Lateral region of the third valvula: entity
(0) is_a sclerite
Medial region of the third valvula: shape
(0) concave in posterior view
Median region of the third valvula: entity
(0) is_a conjunctiva
T9: shape
(0) quadrangular in lateral view
Dorso-median region of T9: width in dorsal view vs. length in dorsal view
(0) width > length
Anterior ridge of T9: count
(0) present
Anterolateral region of anterior ridge of T9: position
(0) adjacent to anterior margin of T9
Distance between Anterior ridge of T9 and anterior margin of T9: anterior-posterior gradient
(0) increasing

Muscles

Attachment site of S7-first valvula muscle (M1) on S7: position
(0) adjacent to the anterior margin of S7
Attachment site of S7-first valvula muscle (M1) on first valvula: position
(0) dorsal to second valvifer

Attachment site of S7-first valvula muscle on first valvula: position
(0) anterior to attachment site of S7-first valvula muscle on S7
Attachment site of dorsal T8-T9 muscle on T8: position
(0) adjacent to the anterior margin of T8
Attachment site of dorsal T8-T9 muscle on T9: position
(0) adjacent to anterior margin of the dorsomedial region of T9
Attachment site of lateral T8-T9 muscle on T8: position
(0) posterodorsal to the attachment site of dorsal T8-T9 muscle on T8
Attachment site of lateral T8-T9 muscle on T9: position
(0) adjacent to anterior ridge of T9
Attachment site of T8-first valvifer muscle on T8: position
(0) dorsal to the attachment site of lateral T8-T9 muscle on T8
Attachment site of T8-first valvifer muscle on first valvifer: position
(0) adjacent to the ninth tergal condyle of the first valvifer
Attachment site of T8-first valvifer muscle on first valvifer: position
(0) on ventral sclerite of the first valvifer adjacent to the intravalvifer articulation
Attachment site of dorsal T9-second valvifer muscle on T9: position
(0) adjacent to the anterior ridge of T9 and the region of T9 dorsal to and ventral to the anterior ridge of T9
Attachment site of dorsal T9-second valvifer muscle on second valvifer: position
(0) adjacent to the anterior section of the dorsal flange of the second valvifer
Ventral T9-second valvifer muscle: count value
(0) 1
Attachment site of ventral T9-second valvifer muscle on second valvifer: position
(0) posterior area of the second valvifer
Attachment site of ventral T9-second valvifer muscle on T9: position
(0) adjacent to anterior region of anterior ridge of T9
First valvifer-second valvifer muscle: count
(0) present
(1) absent
Attachment site of posterior T9-second valvifer muscle on T9: position
(0) on the dorsal region of T9
Attachment site of posterior T9-second valvifer muscle on second valvifer: position
(0) on the median bridge
First valvifer-genital membrane muscle: shape
(0) fan shaped in dorsal view
Attachment site of first valvifer-genital membrane muscle on first valvifer: position
(0) on the medial side of the first valvifer adjacent to the intervalvifer articulation
Attachment site of first valvifer-genital membrane muscle on genital membrane: position
(0) along the median line of the genital membrane
Attachment site of first valvifer-genital membrane muscle on first valvifer: position
(0) on the medial side of the dorsal sclerite of the first valvifer

Attachment site of posterior second valvifer-second valvula muscle on second valvifer: position
(0) on the medial side of the posterior area of the second valvifer
(1) on the medial side of the posterior area of the second valvifer and the anterior area of the second valvifer
Attachment site of posterior second valvifer-second valvula muscle on second valvula: position
(0) on the processus musculares
T9-genital membrane muscle: count
(0) absent
Lateral T9-second valvifer muscle: count
(0) absent
second valvifer-genital membrane muscle: count
(0) absent
anterior second valvifer-second valvula muscles: count
(0) absent

Discussion

The enormous diversity of ovipositor phenotype in Hymenoptera reflects the manner in which the female wasp finds feasible environments for her developing larvae. Host structure and location, as well as the different ways of storing the ovipositor, are arguably the principal factors driving the structural adaptation of these structures (Quicke et al. 1999, Vilhelmsen 2000, Vilhelmsen and Turrisi 2011). Major morphological characteristics of ceraphronoid ovipositors are most likely related to these influences.

Storage of terebra

The ceraphronoid ovipositor is stored in a horizontal position inside the metasoma, with its ventral part concealed by S7 (Fig. 6E). As the first oviposition movement, the contracting muscles between the apical metasomal tergites and sternites expose the ventral part of the ovipositor by rotating it and the ninth abdominal tergite posteriorly, from the resting, horizontal to the active, vertical position. This movement is common within Apocrita (Fig. 6F; Alam 1953, Copland 1976, Fergusson 1988).

The mechanism of the extension of the terebra from the second valvifer-third valvula complex is shared between Ceraphronoidea and most other Hymenoptera. The movement is facilitated by the posterior second valvifer-second valvula muscles (M9: Figs 1A–F, 2A, B, D, 3A, B, 4B, D–F, 5A, C, D; Vilhelmsen 2000). When the muscles contract they cause the bulb to pivot anteriorly at the basal articulation that is composed of the processus articularis and pars articularis (pra, paa: Figs 2A, 2F). As the bulb pivots, the distal end of the terebra move into an extended active position -

compare Fig. 1B (partially extended) with Fig. 2B (retracted). The terebra is held in the longitudinal axis during oviposition: https://scholarsphere.psu.edu/files/r494vk42v

At the end of the oviposition the terebra is retracted prior to the anterior rotation of the ovipositor/T9 complex into the resting, horizontal position. The retraction of the terebra, unlike its extension, seems to be unique for Ceraphronoidea. In other Hymenoptera the movement is accomplished by the contraction of the vertically oriented anterior second valvifer-second valvula muscle that arises from the anterodorsal margin of the second valvifer and inserts on the distal region of the bulb (King and Copland 1969, 1976, Vilhelmsen 2000, Fergusson 1988, Alam 1953). However, this muscle is absent from Ceraphronoidea, and thus we hypothesize a different mechanism for retracting the terebra. We observed a relatively large muscle arising from S7 and inserting dorsally on the first valvula in Ceraphronoidea (M1: Figs 1B, D, 3A). The position of the site of attachments of the muscle suggest that the S7-first valvula muscle (Figs 1B, 3A) aids in the retraction of the terebra. The presence of muscles arising from S7 and inserting on the first valvula has been reported only in some basal Hymenoptera (Dhillon 1966, Vilhelmsen 2000) and in one braconid species, *Stenobracon deesae* (Alam 1953). The S7-first valvulae muscle has an entirely different configuration in basal Hymenoptera. It arises from along the anterior margin of S7 and inserts on the proximal end of the ventral ramus of the first valvula and presumably contributes to the movement of the first valvula aiding the ventral T9-second valvifer muscle (Vilhelmsen 2000). In *Stenobracon*, however, the S7-first valvulae muscle has a very similar structure to that we reported in Ceraphronoidea (Alam 1953) suggesting the possible involvement of this muscle into the retraction of the terebra. Alam (1953) hypothesized that the muscle is involved in the movement of the median conjunctivae of the first valvulae (dorsal wall of the egg canal) influencing the egg movement along the egg canal. Although the anterior second valvifer-second valvulae muscle is present in all non-ceraphronoid Hymenoptera, it is possible that the S7-first valvulae muscle is involved in the retraction of the terebra in other Apocrita, and that this function is not an evolutionary novelty for Ceraphronoidea.

Egg laying mechanism

The paired first and second valvifers and T9, operated together by the dorsal and ventral T9-second valvifer muscles, form the ovipositor machinery that is responsible for "drilling" the terebra into a substrate and moving the egg along the egg canal (Vilhelmsen 2000).

As the dorsal T9-second valvifer muscle (M5: Figs 1A, B, D, E, 2A, B, D, 3B, 4A, B, D, E, F, 5A–C, 6A) contracts the first valvifer pivots posteriorly (in anterior to the left position) at the intervalvifer articulation while contraction of the antagonistic ventral T9-second valvifer muscle (M6: Figs 1A, B, D, E, 2A, B, D, 3B, 4A, B, D, F, 5A, B, 6A) pulls the first valvifer in the opposite direction (compare the position of the tergo-valvifer (tva) and intervalvifer articulations (iva) on Figures 3A and 3E). As this

movement is what slides the first valvula along the second valvulae, the distance the first valvifer moves determines the distance the first valvula moves. The left and right first valvulae slide back and forth alternately during the alternate contraction of the left and right T9-second valvifer muscle pairs (1vf left, 1vv right: Fig. 3B; Vilhelmsen 2000). This alternate movement is what is underlying the advance of the egg inside the egg canal and the drilling of the terebra into a substrate. The former function is facilitated by the presence of internal, posteriorly oriented cuticular modifications (Austin and Browning 1981) while the latter could be aided by anchoring structures on the first valvulae (Vilhelmsen 2000).

Adaptations effecting the alternate movements and configuration of the first valvulae might be mostly affected by the hardness of the substrate and constraints for fast oviposition. Oviposition into a concealed, and therefore relatively immobile host requires a robust system that has to be strong enough to drill or break the barrier. On the other hand, parasitization of an exposed, mobile but relatively soft host requires fast and perhaps less robust mechanism. Two major egg laying habits have been recorded within Ceraphronoidea: oviposition inside a mobile host and oviposition trough a hard but relatively thin barrier enclosing the host, which has restricted movement (Dessart 1995b, c). *Ceraphron* and numerous *Aphanogmus* species were reported to parasitize free living Diptera larvae (Laborius 1972) whereas most *Dendrocerus* species and some *Aphanogmus* parasitize hosts hidden by the hardened integument of the primary host (Fergusson 1980), the wall of the cocoon (Peter and David 1990, Alam 1985) or galls (Bakke 1955) developed around the host.

The relative distance between the anterior angle of the first valvifer and the intervalvifer articulation (ang, iva: Figs 1B, 2C, E, 3C, E, 4A, E, F, 5A, 6A, C, D) is most probably positively correlated with the degree of the sliding motion of the first valvula (Prentice 1998). The posterior margin of the first valvifer angled at the tergo-valvifer articulation (**tva**: Fig.) in most Hymenoptera (Oeser 1961, Vilhelmsen 2000). It is easy to see that the distance between the anterior angle of the first valvifer and the intervalvifer articulation and thus the degree of motion of the first valvula is larger with less acute angle at the tergo-valvifer articulation. A straight posterior margin of the first valvifer has been reported in a few Chalcidoidea (*Epidinocarsis*, Le Ralec et al. 1996; *Spalangia*, fig. 6 in Copland and King 1972) and in Apoidea (*Pryonx*, Prentice 1989). The only ceraphronoid taxon with a angled margin is *Dendrocerus*, while the rest have straight or concave (*Trassedia*) posterior margins. The location of the tergo-valvifer articulation on the posterior margin of the first valvifer seems to be also influenced by the way the first valvifer is moved. The closer the tergo-valvifer articulation is to the intervalvifer articulation and the further from the anterior angle, the further the first valvula will slide on the second valvula. The tergo-valvifer articulation is adjacent to the anterior angle of the first valvifer in *Aphanogmus* sp1. This indicates a first valvula sliding motion of very short distance but, considering the extended site of origin of the T9-second valvifer muscles, with relatively great power. The presence of an anterior angle corresponding to the tergo-valviferal articulation is unique in Hymenoptera.

Two ridges/apodemes are present on T9 in most Hymenoptera, the anterior flange of T9 and the cordate apodeme. The anterior flange extends along the anterior margin of the tergite and might be homologous with the antecosta of the ninth abdominal tergum of other insects because it receives the site of attachment of the dorsal T8-T9 muscle in *Macroxyela* (Vilhelmsen 2000). The dorsal T9-second valvifer muscle arises at least partly from the flange in the rest of Hymenoptera. The cordate apodeme is close to the tergo-valvifer articulation and receive the site of attachment of the ventral T9-second valvifer muscle. It is apophysis-like and extends internally in most basal hymenopterans but ridge-like and extended posteriorly in Siricoidea, Orussidae, and numerous Apocrita ("diagonal ridge" sensu Fergusson 1988). The apodeme is usually well separated from the anterior flange of T9 in Apocrita, except in *Bruchophagus*, where they are seemingly fused anteriorly (Copland and King 1971). Only one ridge, the anterior ridge of T9 extends along the anterior margin of T9 and receives the site of attachment of both the ventral and the dorsal T9-second valvifer muscles in Ceraphronoidea. This condition is unique in Hymenoptera. As described above, the dorsal and ventral T9-second valvifer muscles move the first valvifer indirectly. Host relationships of *Megaspilus* remain unknown, but it is possible that some bands of the ventral muscle insert on the interarticular ridge of the first valvula and thus the contraction of it might cause the direct movement of the sclerite.

The first valvifer is composed of two articulating sclerites in *Trassedia*. Although this condition is possibly plesiomorphic for Insecta (Klass et al. 2013) it is present only in a few taxa i.e. Archaeognatha, most ovipositor bearing Odonata and some Dermaptera (Klass *personal communication*). When the ventral T9-second valvifer muscle contracts the two sclerites of the first valvifer pivot anteriorly together as one unit. The two sclerites articulate with one another anteriorly, however, allowing the dorsal sclerite to pivot posteriorly on the ventral sclerite at the intravalvifer articulation when the dorsal T9-second valvifer muscle is contracted (Figs 3A–E). The first valvulae are thus enabled to slide a very long distance along the second valvulae in this unique system, probably allowing the egg to move quickly down the length of the ovipositor.

The presence or absence of annuli at the tip of the ovipositor may depend on the hardness of the substrate into which the wasp is ovipositing, as well as the circumstances under which oviposition is taking place (Quicke et al. 1999, Le Ralec et al. 1996, Gerling et al. 1998). Megaspilidae, similar to numerous other non-ichneumonoid apocritans do have annuli apically only on the second valvulae whereas Ceraphronidae lack them from both valvulae. In general it seems that the harder the substrate is, the more developed the ovipositor sculpture is (Le Ralec et al. 1996).

A minute gland (dgl?: Figs 3B, 5A) and a relatively larger gland reservoir (res: Figs 3A–E, 5A, B, E, F, 4C), enclosed by the second valvifers, have been detected in Ceraphronidae including *Trassedia* and *Lagynodes*. The Dufour's gland and the venom gland reservoir has a similar location in some Chalcidoidea (Copland and King 1971) where it was hypothesized that the ventral second T9-second valvifer muscles might aid to discharge the reservoir (res: Fig. 5B). The gland extract of this possible venom reservoir is resin-like (hard, transparent and amber colored) in critical point dried *Cer-*

aphron specimens (res: Fig. 5F) implying its possible cement nature that might be used for coating the eggs or fastening them on a surface. *Ceraphron* and some *Aphanogmus* species are reported to be endoparasitoids (Cordero and Cave 1992) in which lifestyle the egg coating can be crucial for avoiding the host immune response. We did not observe any glands or resin containing reservoirs in Megaspilinae. Höller et al. (1993) identified the Dufour's gland in *Dendrocerus carpenteri* outside of the second valvifers and reported the absence of the venom gland in this taxon. Nevertheless, more accurate, TEM based examination of the accessory gland system of Ceraphronoidea is needed for clarifying the function of the gland and gland reservoir located inside the second valvifer.

In general, the ovipositors of *Ceraphron*, *Trassedia* and *Aphanogmus* sp. 2 are less robust and capable of a very large degree of motion, corresponding to the available data about ovipositing in exposed and active hosts. *Trassedia* represents, perhaps, a more extreme version of the "quickly into soft substrate" oviposition type. Megaspilidae, on the other hand, have a stronger, more robust ovipositor systems, which afford the smaller degree of motion required for handling a harder substrate concealing a static host. *Dendrocerus*, for example, exhibits extended sites of origins for muscles and a very small degree of motion for the first valvulae. *Aphanogmus* sp. 1, although it belongs to Ceraphronidae, shares numerous characteristics with *Dendrocerus* and therefore may represent the *Aphanogmus*-group that parasitizes hosts obscured by harder barrier, e.g., the wall of a plant gall, and thus is a case of parallelism driven by the same environmental constraints.

So far it is widely accepted that Ceraphronoidea is composed of two extant families, Ceraphronidae and Megaspilidae, plus two fossil families not treated here. The limits between the two families, however, have been challenged recently (Mikó and Deans 2009, Mikó et al. in press). Although the presence of the annuli in Megaspilidae and absence from Ceraphronidae supports the traditional classification, the location of a resin producing gland inside the second valvifers is shared by the megaspilid subfamily Lagynodinae and Ceraphronidae.

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Appendix

Anatomical terms used, cross-referenced to an ontological (formal) definition (Hymenoptera Anatomy Ontology; URI = Uniform Resource Identifier).

Abbreviation	Label	Concept	URI
	absent	A quality denoting the lack of an entity.	http://purl.obolibrary.org/obo/PATO_0000462
	adjacent to	A spatial quality inhering in a bearer by virtue of the bearer's being located near in space in relation to another entity.	http://purl.obolibrary.org/obo/PATO_0002259
ann	annulus, annuli	The carina that is transverse and extends across the lateral wall of the terebra.	http://purl.obolibrary.org/obo/HAO_0001173
ang	anterior angle of the first valvifer	The corner on the first valvifer that marks the posterior end of the first valvula.	http://purl.obolibrary.org/obo/HAO_0002168
aa	anterior area of the second valvifer	The area of the second valvifer which is anterior to the anatomical line that is the shortest distance from the first valviferal fossa of the second valvifer and the ventral margin of the second valvifer	http://purl.obolibrary.org/obo/HAO_0002169
	anterior flange of T9	The flange that extends along the anterolateral margin of female T9.	http://purl.obolibrary.org/obo/HAO_0001171
af1	anterior flange of the first valvifer	The flange that extends anteriorly on the first valvifer and overlaps with the posterior margin of the anterior area of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0002166
	anterior margin	anatomical margin and (overlaps some anterior side)	http://purl.obolibrary.org/obo/BSPO_0000671
an2	anterior notch of the dorsal valve	The notch that is located anteriorly on the dorsal ramus of the second valvula that accommodates the ventral ramus of the second valvula and the first valvula.	http://purl.obolibrary.org/obo/HAO_0002178
	anterior region, anteriorly	anatomical region and (overlaps some anterior side)	http://purl.obolibrary.org/obo/BSPO_0000071
ar9	anterior ridge of T9	The ridge that extends along the anterior margin of female T9 and receives the site of origin of the ventral and the dorsal T9-second valvifer muscles.	http://purl.obolibrary.org/obo/HAO_0002182
	anterior second valvifer-second valvula muscle	The ovipositor muscle that arises from the anterodorsal part of the second valvifer and inserts subapically on the processus articulares.	http://purl.obolibrary.org/obo/HAO_0001166
asf	anterior section of dorsal flange of the second valvifer	The area of the dorsal flange of the second valvifer that is anterior to the site of origin of the basal line.	http://purl.obolibrary.org/obo/HAO_0002173
	anterior to	A spatial quality inhering in a bearer by virtue of the bearer's being located toward the front of an organism relative to another entity.	http://purl.obolibrary.org/obo/PATO_0001632

Abbreviation	Label	Concept	URI
	anterodorsal margin	anatomical margin and (overlaps some anterior side) and (overlaps some dorsal side)	http://purl.obolibrary.org/obo/BSPO_0000686
	anterolateral region	Anatomical region and (overlaps some anterior side) and (overlaps some lateral side)	http://purl.obolibrary.org/obo/BSPO_0000029
	apodeme	The process that is internal.	http://purl.obolibrary.org/obo/HAO_0000142
	attachement site	The area of the integument where muscles are attached to epidermal cells.	http://purl.obolibrary.org/obo/HAO_0002184
	banding pattern	The anatomical cluster that is composed of the strongly sclerotised areas corresponding with the annuli and less strongly sclerotised areas situated between them.	http://purl.obolibrary.org/obo/HAO_0001176
	basal articulation	The articulation that is part of the second valvifer-second valvula-third valvula complex and adjacent to the rhachis.	http://purl.obolibrary.org/obo/HAO_0001177
bl	basal line of the second valvifer	The line on the second valvifer that extends between the pars articularis and the dorsal flange of second valvifer.	http://purl.obolibrary.org/obo/HAO_0002171
	bent	A shape quality inhering in a bearer by virtue of the bearer's having one or more angle(s) in its length.	http://purl.obolibrary.org/obo/PATO_0000617
	blunt	A shape quality inhering in a bearer by virtue of the bearer's terminating gradually in a rounded end.	http://purl.obolibrary.org/obo/PATO_0001950
bulb	bulb	The anterior area of the dorsal valve that is bulbous.	http://purl.obolibrary.org/obo/HAO_0002177
	concave	A shape quality in a bearer by virtue of the bearer's curving inward.	http://purl.obolibrary.org/obo/PATO_0001857
	conjunctiva	The area of the integument that is weakly sclerotized, with thin exocuticle.	http://purl.obolibrary.org/obo/HAO_0000221
	convex	A shape quality that obtains by virtue of the bearer having inward facing edges; having a surface or boundary that curves or bulges outward, as the exterior of a sphere.	http://purl.obolibrary.org/obo/PATO_0001355
	cordate apodeme	The apodeme on the anterior margin of the abdominal tergum 9 that receives the ventral T9-second valvifer muscle.	http://purl.obolibrary.org/obo/HAO_0001585
	corner	The projection that is located at the intersection of two or more edges.	http://purl.obolibrary.org/obo/HAO_0000223
	count	The number of entities of this type that are part of the whole organism.	http://purl.obolibrary.org/obo/PATO_0000070
	count value		http://purl.obolibrary.org/obo/PATO_0000416
	curvature	A surface shape quality inhering in a bearer by virtue of the bearer's exhibiting a degree of bending.	http://purl.obolibrary.org/obo/PATO_0001591

Abbreviation	Label	Concept	URI
	curved dorsally	A curvature quality inhering in a bearer by virtue of the bearer's being curved towards the back or upper surface of an organism.	http://purl.obolibrary.org/obo/PATO_0001468
	curved medially	A curvature quality inhering in a bearer by virtue of the bearer's being curved towards the middle.	http://purl.obolibrary.org/obo/PATO_0002164
	distal notch of the dorsal valve	The notch that is distal on the dorsal valve.	http://purl.obolibrary.org/obo/HAO_0002179
	distal region	Anatomical region and (overlaps some distal side)	http://purl.obolibrary.org/obo/BSPO_0000078
dvc	distal vertical conjunctiva of the second valvifer-third valvifer complex	The conjunctiva that traverses the second valvifer-third valvula complex and is located distal to the median bridge of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0002180
	distance	A quality that is the extent of space between two entities.	http://purl.obolibrary.org/obo/PATO_0000040
	dorsal margin	Anatomical margin and (overlaps some dorsal side).	http://purl.obolibrary.org/obo/BSPO_0000679
dp	dorsal projection of the second valvifer	The projection that is located on the second valvifer and corresponds to the proximal end of the rachis.	http://purl.obolibrary.org/obo/HAO_0002172
	dorsal region	anatomical region and (overlaps some dorsal side)	http://purl.obolibrary.org/obo/BSPO_0000079
d1vf	dorsal sclerite of the first valvifer	The sclerite of the first valvifer that is located dorsally of the transvalviferal conjunctiva.	http://purl.obolibrary.org/obo/HAO_0002163
M2	dorsal T8-T9 muscle	The abdominal muscle that arises from the anteromedian margin of female T8 and inserts on the anteromedian margin of the female T9.	http://purl.obolibrary.org/obo/HAO_0001571
M5	dorsal T9-second valvifer muscle	The ovipositor muscle that arises along the posterodorsal part of the anterior margin of female T9 and inserts on the anterior section of the dorsal flanges of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0001569
	dorsal to	x dorsal_to y if x is further along the dorso-ventral axis than y, towards the back. A dorso-ventral axis is an axis that bisects an organism from back (e.g. spinal column) to front (e.g. belly).	http://purl.obolibrary.org/obo/BSPO_0000098
	dorsal valve	The area that is articulated with the right and left second valvifers at the basal articulation and bears the rhachies.	http://purl.obolibrary.org/obo/HAO_0001658
	dorsal view		http://purl.obolibrary.org/obo/BSPO_0000063
	dorsomedial region	anatomical region and (overlaps some dorsal side) and (overlaps some medial side)	http://purl.obolibrary.org/obo/BSPO_0000069

Abbreviation	Label	Concept	URI
ec	egg canal	The anatomical space that is between the left and right rhachises.	http://purl.obolibrary.org/obo/HAO_0002191
	elongated	A quality inhering in a bearer by virtue of the bearer's length being notably higher than its width.	http://purl.obolibrary.org/obo/PATO_0001154
	fan-shaped	A quality inhering in a bearer that is shaped in the form of a fan.	http://purl.obolibrary.org/obo/PATO_0002219
1vf	first valvifer	The area of the first valvifer-first valvula complex that is proximal to the aulax, bears the ninth tergal condyle of the first valvifer and the second valviferal condyle of the first valvifer and is connected to the genital membrane by muscle.	http://purl.obolibrary.org/obo/HAO_0000338
M8	first valvifer-genital membrane muscle	The ovipositor muscle that arises from the posterior part of the first valvifer and inserts anteriorly on the genital membrane anetrior to the T9-genital membrane muscle.	http://purl.obolibrary.org/obo/HAO_0001746
	first valvifer-second valvifer muscle	The ovipositor muscle that arises from the first valvifer and inserts on the second valvifer.	http://purl.obolibrary.org/obo/HAO_0002189
1vv	first valvula, first valvulae	The area of the first valvifer-first valvula complex that is delimited distally by the proximal margin of the aulax.	http://purl.obolibrary.org/obo/HAO_0000339
gm	genital membrane	The conjunctiva that connects the ventral margins of the second valvifers.	http://purl.obolibrary.org/obo/HAO_0001757
	height	A 1-D extent quality inhering in a bearer by virtue of the bearer's vertical dimension of extension.	http://purl.obolibrary.org/obo/PATO_0000119
	increasing	quality and (increased_in_magnitude_relative_to some normal)	http://purl.obolibrary.org/obo/PATO_0002300
iar	interarticular ridge of the first valvifer	The ridge that extends along the posterior margin of the first valvifer between the intervalvifer and tergovalvifer articulations.	http://purl.obolibrary.org/obo/HAO_0001562
iva	intervalvifer articulation	The articulation between the first valvifer and second valvifer.	http://purl.obolibrary.org/obo/HAO_0001558
iava	intravalvifer articulation	The articulation between the dorsal sclerite of the first valvifer and the ventral sclerite of the first valvifer.	http://purl.obolibrary.org/obo/HAO_0002165
	lateral region	anatomical region and (overlaps some lateral side)	http://purl.obolibrary.org/obo/BSPO_0000082
M3	lateral T8-T9 muscle	The ninth abdominal tergal muscle that arises from the anterolateral margin of female T8 and inserts on the anterolateral margin of female T9.	http://purl.obolibrary.org/obo/HAO_0001776
	lateral T9-second valvifer muscle	The muscle that arises from the posteroventral parts of the female T9 and inserts on the median bridge.	http://purl.obolibrary.org/obo/HAO_0002187

Abbreviation	Label	Concept	URI
	lateral view		http://purl.obolibrary.org/obo/BSPO_0000066
	length of anterior area of second valvifer	The anatomical line that is parallel with the longitudinal body axis and the shortest among the anatomical lines that extends between the anterior and posterior margins of the anterior area of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0002240
	length of dorsal projection	The anatomical line that is parallel with the longitudinal body axis and the shortest among the anatomical lines that extends between the anterior and posterior margins of the dorsal projection.	http://purl.obolibrary.org/obo/HAO_0002193
	length of female T9	The anatomical line that is parallel with the longitudinal body axis and the shortest among the anatomical lines that extend between the anterior and posterior margins of female T9.	http://purl.obolibrary.org/obo/HAO_0002241
	medial side	a point in the centre of the organism (where the left-right axis intersects the midsagittal plane)	http://purl.obolibrary.org/obo/BSPO_0000067
	median bridge	The area that connects posterodorsally the second valvifers and is the site of attachment for the posterior T9-second valvifer muscle.	http://purl.obolibrary.org/obo/HAO_0001780
mc2	distal notch of the dorsal valve	The notch that is distal on the dorsal valve.	http://purl.obolibrary.org/obo/HAO_0002179
mc1	medial conjunctiva of the first valvulae	The conjunctiva that extends medially along the first valvula.	http://purl.obolibrary.org/obo/HAO_0002192
	median line	An axis that bisects an organism from head end to opposite end of body or tail.	http://purl.obolibrary.org/obo/BSPO_0000013
	medial region	anatomical region and (overlaps some medial side)	http://purl.obolibrary.org/obo/BSPO_0000083
	ninth tergal condyle of the first valvifer	The condyle that is located on the first valvifer and articulates with the first valviferal fossa of T9.	http://purl.obolibrary.org/obo/HAO_0002160
oth	olistheters	The anatomical cluster that is composed of the rhachis of the second valvula and the aulax of the first valvula.	http://purl.obolibrary.org/obo/HAO_0001103
	overlaps	x overlaps y if they have some part in common.	http://purl.obolibrary.org/obo/bspo%23overlaps
	ovipositor	The anatomical cluster that is composed of the first valvulae, second valvulae, third valvulae, first valvifers, second valvifers and female T9.	http://purl.obolibrary.org/obo/HAO_0000679
paa	pars articularis	The articular surface that is situated anteriorly on the ventral margin of the second valvifer and forms the lateral part of the basal articulation.	http://purl.obolibrary.org/obo/HAO_0001606

Abbreviation	Label	Concept	URI
	position	A spatial quality inhering in a bearer by virtue of the bearer's spatial location relative to other objects in the vicinity.	http://purl.obolibrary.org/obo/PATO_0000140
	posteriorly	anatomical gradient and (has_axis some anterior/posterior axis)	http://purl.obolibrary.org/obo/BSPO_0000052
pa	posterior area of the second valvifer	The area of the second valvifer that is posterior to the anatomical line that is the shortest distance from the first valviferal fossa of the second valvifer to the ventral margin of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0002170
	posterior margin	anatomical margin and (overlaps some posterior side)	http://purl.obolibrary.org/obo/BSPO_0000672
p1v	posterior margin of first valvifer	The margin of the first valvifer that is posterior and extends between the intervalvifer articulation and the anterior angle of the first valvifer.	http://purl.obolibrary.org/obo/HAO_0002159
M9	posterior second valvifer-second valvula muscle	The ovipositor muscle that arises posteroventrally from the second valvifer and inserts on the processus musculares of the second valvula.	http://purl.obolibrary.org/obo/HAO_0001815
psf	posterior section of dorsal flange of the second valvifer	The area of the dorsal flange of the second valvifer that is posterior to the to the site of origin of the basal line.	http://purl.obolibrary.org/obo/HAO_0002174
M7	posterior T9-second valvifer muscle	The ovipositor muscle that arises medially from the posterodorsal part of female T9 and inserts on the median bridge of the second valvifers.	http://purl.obolibrary.org/obo/HAO_0001813
	posterior view		http://purl.obolibrary.org/obo/BSPO_0000056
	postero-medial region	anatomical region and (overlaps some posterior side) and (overlaps some medial side)	http://purl.obolibrary.org/obo/BSPO_0000070
	posterodorsal to	A spatial quality inhering in a bearer by virtue of the bearer's being located toward the rear and upper surface of an organism relative to another entity.	http://purl.obolibrary.org/obo/PATO_0001916
	posteroventral corner of first valvifer	The corner of the first valvifer that is adjacent to the intervalvifer articulation.	http://purl.obolibrary.org/obo/HAO_0002239
	present	A quality inhering in a bearer by virtue of the bearer's existence.	http://purl.obolibrary.org/obo/PATO_0000467
pra	processus articularis	The process that extends laterally from the proximal region of the second valvula and forms the median part of the basal articulation, and corresponds to the site of attachment for the anterior second valvifer-second valvula muscle.	http://purl.obolibrary.org/obo/HAO_0001704

Abbreviation	Label	Concept	URI
prm	processus musculares	The apodeme that extends dorsally from the proximal part of the second valvula to the genital membrane and receives the site of attachment of the posterior second valvifer-second valvula muscle.	http://purl.obolibrary.org/obo/HAO_0001703
	proportion	A quality inhering in a bearer by virtue of the bearer's magnitude in respect to a related entity.	http://purl.obolibrary.org/obo/PATO_0001470
	quadrangular	A shape quality inhering in a bearer by virtue of the bearer's having four angles and four sides.	http://purl.obolibrary.org/obo/PATO_0001988
	region	A 3D region in space without well-defined compartmental boundaries; for example, the dorsal region of an ectoderm.	http://purl.obolibrary.org/obo/BSPO_0000070
	resinous	A physical quality inhering in a bearer by virtue of the bearer exhibiting molecular attraction to another entity in contact.	http://purl.obolibrary.org/obo/PATO_0002331
	ridge	The apodeme that is elongate.	http://purl.obolibrary.org/obo/HAO_0000899
	S7	The sternite that is connected to the first valvula via muscles.	http://purl.obolibrary.org/obo/HAO_0002185
	S7-first valvula muscle	The muscle that originates from the abdominal sternum 7 and inserts on the first valvula.	http://purl.obolibrary.org/obo/HAO_0001668
	sclerite, sclerites	The area of the integument where the cuticle is well sclerotised with thick exocuticle.	http://purl.obolibrary.org/obo/HAO_0000909
	second valvifer-genital membrane muscle	The ovipositor muscle that arises anteriorly from the dorsal flange of the second valvifer and inserts anteriorly on the dorsal part of the genital membrane.	http://purl.obolibrary.org/obo/HAO_0001672
2vf	second valvifer	The area of the second valvifer-second valvula-third valvula complex that is proximal to the basal articulation and to the processus musculares and articulates with female T9.	http://purl.obolibrary.org/obo/HAO_0000927
	second valvifer-genital membrane muscle	The ovipositor muscle that arises anteriorly from the dorsal flange of the second valvifer and inserts anteriorly on the dorsal part of the genital membrane.	http://purl.obolibrary.org/obo/HAO_0001672
	second valviferal condyle of the first valvifer	The condyle that is located on the first valvifer and articulates with the first valviferal fossa of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0002167
	second valvifer-third valvula complex	The area of the second valvifer-second valvula-third valvula complex that is proximal to the basal articulation.	http://purl.obolibrary.org/obo/HAO_0002181
2vv	second valvula, second valvulae	The area of the second valvifer-second valvula-third valvifer complex that is distal to the basal articulation and to the processus musculares and is limited medially by the median body axis.	http://purl.obolibrary.org/obo/HAO_0000928

Abbreviation	Label	Concept	URI
spa	sensillar patch	The patch that is composed of placoid sensilla adjacent to the intervalvifer articulation.	http://purl.obolibrary.org/obo/HAO_0001671
	shape	A morphological quality inhering in a bearer by virtue of the bearer's ratios of distances between its features (points, edges, surfaces and also holes etc).	http://purl.obolibrary.org/obo/PATO_0000052
	sharpness	A shape quality inhering in a bearer by virtue of the bearer's having a sharp or tapered end or point.	http://purl.obolibrary.org/obo/PATO_0000944
	sharp	A shape quality inhering in a bearer by virtue of the bearer's terminating in a point or edge.	http://purl.obolibrary.org/obo/PATO_0001419
	spatulate	A shape quality inhering in a bearer by virtue of the bearer's being oblong, with the lower end very much attenuated.	http://purl.obolibrary.org/obo/PATO_0001937
	straight	A shape quality inhering in a bearer by virtue of the bearer's being free of curves, bends, or angles.	http://purl.obolibrary.org/obo/PATO_0002180
	surrounds		http://purl.obolibrary.org/obo/BSPO_0000101
	T8	The tergite that is connected to female T9 by muscles.	http://purl.obolibrary.org/obo/HAO_0002188
	T8-first valvifer muscle	The ovipositor muscle that originates from the lateral part of female T8 and inserts on the dorsal margin of the first valvifer.	http://purl.obolibrary.org/obo/HAO_0001640
	T9	The tergite that is articulted with the first valvifer and second valvifer and is connected to the second valvifer via muscles.	http://purl.obolibrary.org/obo/HAO_0000075
	T9-genital membrane muscle	The ovipositor muscle that arises from the cordate apodeme and inserts dorsally on the proximal part of the genital membrane and on the opposite cordate apodeme.	http://purl.obolibrary.org/obo/HAO_0001639
	tapered	A shape quality inhering in a bearer by virtue of the bearer's being gradually narrower or thinner toward one end.	http://purl.obolibrary.org/obo/PATO_0001500
trb	terebra	The anatomical cluster that is composed of the first and second valvulae.	http://purl.obolibrary.org/obo/HAO_0001004
tva	tergo-valvifer articulation	The articulation that is located between the abdominal tergum 9 and the first valvifer and is composed of the ninth tergal condyle of the first valvifer and the first valviferal fossa of the ninth tergite.	http://purl.obolibrary.org/obo/HAO_0001636
	thickened	A thickness which is relatively high.	http://purl.obolibrary.org/obo/PATO_0000591
	thickness	A 1-D extent quality which is equal to the dimension through an object as opposed to its length or width.	http://purl.obolibrary.org/obo/PATO_0000915

Abbreviation	Label	Concept	URI
3vv	third valvula, third valvulae	The area of the second valvifer-third valvula complex that is posterior to the distal vertical conjunctiva of the second valvifer-third valvula complex.	http://purl.obolibrary.org/obo/HAO_0001012
tvc	transvalvifer conjunctiva	The conjunctiva that traverses the first valvifer and separates the dorsal and ventral sclerites of the first valvifer.	http://purl.obolibrary.org/obo/HAO_0002162
	triangular	A shape quality inhering in a bearer by virtue of the bearer's having three angles.	http://purl.obolibrary.org/obo/PATO_0001875
res	venom gland reservoir of the second valvifer	The gland reservoir that is between the second valvifers.	http://purl.obolibrary.org/obo/HAO_0002176
	ventral margin	anatomical margin and (overlaps some ventral side)	http://purl.obolibrary.org/obo/BSPO_0000684
vr2	ventral ramus of the second valvula	The area of the second valvifer-second vavula-third valvifer complex that bears the rhachis.	http://purl.obolibrary.org/obo/HAO_0001107
v1v	ventral sclerite of the first valvifer	The sclerite of the first valvifer that is ventral to the transvalviferal conjunctiva.	http://purl.obolibrary.org/obo/HAO_0002164
M6	ventral T9-second valvifer muscle	The ovipositor muscle that arises from the lateral region of female T9 and inserts along the posterior part of the dorsal flange of the second valvifer.	http://purl.obolibrary.org/obo/HAO_0001616
	ventral to	x ventral_to y if x is further along the dorso-ventral axis than y, towards the front. A dorso-ventral axis is an axis that bisects an organism from back (e.g. spinal column) to front (e.g. belly).	http://purl.obolibrary.org/obo/BSPO_0000102
	width	A 1-D extent quality which is equal to the distance from one side of an object to another side which is opposite.	http://purl.obolibrary.org/obo/PATO_0000921